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Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. I. Solubilizing Gluten and Flour Protein[1]

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ABSTRACT

Gluten proteins, after solubilization in 0.005N lactic acid, were fractionated by precipitation at various pH levels with 0.1N sodium carbonate. In general, as pH increased for a given fraction, glutenins decreased and gliadins increased. The pH 6.1-soluble protein contained little or no glutenins but high concentrations of gliadins. The decrease in glutenins and increase in gliadins, as pH increased, were accompanied by a consistently large decrease in mixing time and baking absorption and an increase in oxidation requirement. Also, loaf volume of a protein fraction increased with higher pH values. The ratio of gliadin to glutenin in the pH 6.1-insoluble fraction was more nearly optimum for loaf volume than that in any of the other fractions or the crude gluten.

Protein in wheat flour also was fractionated by water-solubilizing techniques. Extracted protein increased from 6.5% for one extraction to 37.6% of total flour protein for eight consecutive water extractions. The ratio of gliadins to other proteins increased markedly with each consecutive water extraction. Water-insoluble protein and starch fractions each contained the spectrum of proteins including glutenins, gliadins, and the rapidly moving bands. Evidence of protein interaction was obtained when the water-soluble protein and water-insoluble protein fractions were reconstituted and baked into bread.

A dough moulder and other breadmaking equipment for processing 10 g. of flour are described.

That wheat flour gluten is the fraction of wheat flour basically responsible for wheat quality was demonstrated by Finney (1); he fractionated wheat flours of differing quality, exchanged the glutens, reconstituted them with the starch plus water-soluble materials, and baked them into bread. By reconstituting the original flours as controls, he demonstrated that his fractionation and reconstitution techniques did not damage wheat proteins. Obviously the next step was to fractionate gluten protein. The purpose of our studies was to fractionate gluten protein by solubilizing it in lactic acid at various pH levels, fractionate flour protein by water-solubilizing techniques, and evaluate the fractions by breadmaking and starch-gel electrophoresis.

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MATERIALS AND METHODS

Flours

Regional baking standard (RBS) was a blend of many varieties of hard winter wheats harvested at many stations in the central and southern Great Plains. It had a protein content of 12.8%, medium physical dough properties, and good loaf-volume potential. We used two other composites of hard winter wheat varieties that possessed good loaf-volume potential: Comanche, with medium-strong, and C.I. 12995 (Quivira-Tenmarq x Marquillo-Oro), with strong physical dough properties.

Fractionating Flour into Gluten, Starch, and Water-Solubles

We used the following procedure at room temperature (25°C.): Flour (250 g. at 4°C.) was slurried for 1 min. in a 2-liter Erlenmeyer flask with 500 cc. of distilled water (4°C.). After the flask was rinsed with an additional 100 cc. of distilled water, combined slurry and rinse water were centrifuged for 20 min. at about 1,000 x g. After the supernatant and centrifugate were combined, the gluten was washed out by hand. After the gluten had been washed until it became a smooth, coherent mass (20 to 30 min. of gentle manipulation), the starch and wash water were poured through a stainless-steel sieve with openings of 0.0053 in. Then the gluten was rinsed by manipulation for 3 to 5 min. with each of six successive 50-ml. portions of distilled water. Rinse water was combined with the previously obtained starch and wash water.

The wet crude gluten was placed in a closed jar and allowed to relax, 4 hr. for Comanche and 5 hr. for C.I. 12995. The wet gluten was then frozen and lyophilized to about 3% moisture. Starch and water-solubles were shelled by freezing in concentric layers on the inside wall of a 1-gal. bottle, and then lyophilized to give a combined starch and water-soluble fraction. Lyophilized starch and water-solubles were rehydrated to about 13% moisture, and ground in a mortar to pass a 115-mesh Tyler sieve. Lyophilized gluten and its fractions were ground in a micro Wiley mill to pass a 60-mesh sieve. All fractions, after being analyzed for moisture and protein content, were stored at −20°C.

Fractionating Gluten Proteins at Various pH Levels

We followed this procedure at 25°C.: Wet gluten was scissored into small pieces and gently stirred for 5 hr. in 700 ml. of 0.005N lactic acid. The gluten solubilized at about pH 4.7 was centrifuged to remove the lactic acid-insoluble material prior to adjustment to pH levels of 5.6, 5.8, and 6.1 with 0.1N sodium carbonate. Gluten precipitated at each pH level was recovered by centrifugation at 1,000 x g prior to adjustment of the supernatant to the next higher pH level. Each centrifugate, including the pH 4.7-insoluble fraction, was adjusted to pH 6.1. Gluten solubilized at pH 4.7 also was neutralized to pH 6.1 without removal of the insoluble material (one-step precipitation). All protein fractions, after being lyophilized, ground, and analyzed for moisture and protein content, were reconstituted singly and in their original proportions with the starch plus water-soluble material, and baked into bread. All flours were reconstituted to the protein contents of the original flours.

Water-Solubility of Flour Proteins

Flour (250 g.) was slurried with 1,200 ml. of distilled water, stirred for 30 min. under an atmosphere of nitrogen, and centrifuged only enough to make decantation

feasible (300 revolutions in 1½ min. in an International size 2). The decanted liquid was replaced with an equal amount of distilled water (about 850 ml.), and the loose centrifugate was resuspended and stirred 30 min. After seven extractions, the residue was separated into a starch-rich fraction (S) and a water-insoluble, protein-rich fraction (WIP) by washing over a 10xx nylon sieve. The wash water was considered to be the eighth extraction. When eight similar extractions were made without separating the starch-rich and protein-rich fractions, the residue was designated WIP-S (water-insoluble protein fraction plus starch). Samples from each extraction were analyzed for Kjeldahl protein. The supernatants (extractions) were designated successively as A, B, C, D, E, F, G, and H. Fractions A through H collectively were designated water-soluble protein fractions (WSP).

All fractions were lyophilized, ground, and analyzed for protein and moisture. Fractions were reconstituted singly and in various combinations with starch (S) and water-insoluble protein plus starch (WIP-S) and baked into bread (10 g. of flour). All fractions were characterized by starch-gel electrophoresis.

The WSP, WIP, and S fractions also were reconstituted as follows before being baked into bread: (a) dry WIP-S was reconstituted with dry WSP; (b) the WIP-S, while still wet, was reconstituted with dry WSP; (c) wet WIP and dry WSP from 50 g. of RBS flour were solubilized in 140 ml. of 0.005N lactic acid, adjusted to pH 6.1 with 0.1N sodium carbonate, lyophilized, and reconstituted with the dried starch residue.

Starch-Gel Electrophoresis

Each fraction was dissolved in pH 3.2 aluminum lactate and lactic acid buffer containing 3M urea, and fractionated by vertical starch-gel electrophoresis. Separations of 0.050 ml. of a 10% protein dispersion were made. Acid-hydrolyzed potato starch (from Connaught Medical Research Laboratory, Toronto, Ontario), 15% more than manufacturer's recommendation of 10.1 to 10.6%, was gelatinized in buffered 3.0M urea. Electrophoresis was performed at 30 ma. and approximately 325 volts for 6 hr. at room temperature. The gel preparations were sliced and stained in 0.1% Amido Black 10B, and excess stain was removed with several changes of distilled water.

The Ten-Gram Baking Method

The method employed optimum mixing time, water absorption, and potassium bromate (10 to 60 p.p.m.), and a formula that included: flour 10 g., sugar 0.6 g., salt 0.15 g., shortening 0.3 g., yeast 0.2 g., nonfat dry milk 0.4 g., and 60° L. malt syrup 0.05 g. Doughs were fermented 3 hr. and proofed 55 min. at 30°C. Additional related details are given by Finney and Barmore (2,3,4).

The equipment for the 10-g. bake test included bread pans with these inside dimensions: bottom 23 by 51 mm., top 32 by 60 mm., and height 23 mm. The stops and guards on National sheeting rolls were removed, and the distance between rolls was set at 2.5 mm. for sheeting the dough. The dough moulder consisted of a fixed wood base and a plastic and wood sled (Fig. 1). The base was 3.5 by 41 by 1,000 mm. The sled (without base) had a front opening 17 by 41 mm., a back opening 14 by 41 mm., and length 285 mm. After the dough was sheeted into a ribbon, it was laid on the wooden base and rolled (curled) enough at one end (by hand) to make contact with the sled. Moving the sled along the base rolled the

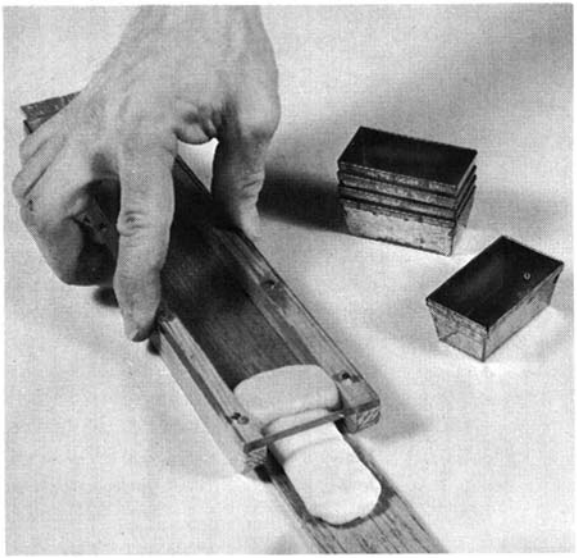


Fig. 1. Moulder and bread pans for doughs made from 10 g. of flour.

dough and compressed it into a cylinder. Baking time was 15 min. and temperature 218°C. Loaf volume was measured by rapeseed displacement.

The correlation coefficient of 100-g. vs. 10-g. loaf volumes was 0.98, significant far beyond the 0.1% level. Loaf volume of 10 g. of flour multiplied by 11.5 approximately equaled that of 100 g. of flour. Optimum absorptions of 10-g. doughs usually were higher than those of 100-g. doughs. Crumb grains of 10-g. doughs were comparable to those of 100-g. doughs.

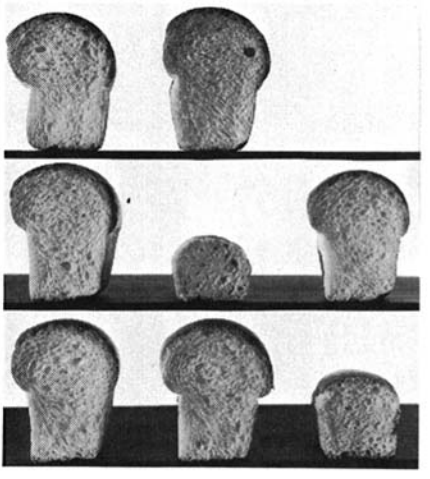
RESULTS AND DISCUSSION

Fractionating Gluten Proteins at Various pH Levels

The pH levels at which the fractions were precipitated, together with protein contents, protein yields (expressed as percent of total flour protein), and baking data for fractions obtained from the varieties Comanche and C.I. 12995 are given in Table I. Photographs of 10-g. loaves of bread baked from reconstituted and control flours of C.I. 12995 are reproduced in Fig. 2. Loaf volumes of reconstituted C.I. 12995 and Comanche flours containing crude gluten, a one-step precipitation of gluten, and all protein fractions, in general, were equal to those of the corresponding control flours.

The pH 4.7-insoluble fraction of gluten contained bran particles and starch not washed from the gluten during the initial flour fractionation. That material, obtained as a paste on the bottom of the centrifuge cup, had no elasticity or cohesiveness. Its slightly gray color contributed some color to the loaf. It apparently contributed little to the loaf volume of the original flour. Its baking absorption was highest of any fraction. During mixing, it appeared not to reach a point of minimum mobility.

The gluten fraction soluble at pH 4.7 and precipitated at pH 5.6 was glutenlike



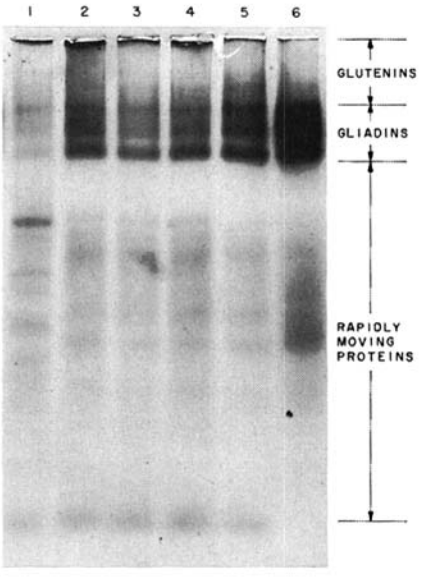


Fig. 2 (left). Cut loaves of bread baked from the flours that were reconstituted with C.I. 12995 protein fractions. Fractions were obtained by precipitating, at various pH levels, the gluten proteins that were solubilized in 0.005N lactic acid. Loaves represent, from left to right: 1st row, original flour and crude gluten; 2nd row, all, pH-4.7 insoluble, and pH-5.6 insoluble fractions; 3rd row, pH-5.8 insoluble, pH 6.1-insoluble, and pH 6.1-soluble fractions.

Fig. 3 (right). Starch-gel electrophoretic patterns of C.I. 12995 gluten and its fractions obtained at various pH levels. Patterns represent: 1, pH 4.7-insoluble; 2, crude gluten; 3, pH 5.6-insoluble; 4, pH 5.8-insoluble; 5, pH 6.1-insoluble; 6, pH 6.1-soluble.

but lacked extensibility. Its baking absorption was not as high as that of the insoluble material, but was higher than those of the other gluten fractions.

The gluten fraction soluble at pH 5.6 and precipitated at pH 5.8 most closely resembled the unfractionated gluten in extensibility and baking properties.

The gluten fraction soluble at pH 5.8 and precipitated at pH 6.1 was very extensible and became sticky and difficult to handle when allowed to relax. Its oxidation requirement was greater than that of the two previously precipitated fractions and the original flour. Loaf volume of bread baked from reconstituted flour, in which the gluten was replaced with that fraction, was higher than that of the corresponding original flour.

The gluten fraction soluble at pH 6.1 was extremely extensible. Its baking absorption was very low and mixing time extremely short. Bread baked from reconstituted flour, in which the gluten protein was replaced with that fraction, was extremely poor.

The crude gluten and fractions of C.I. 12995 were characterized by starch-gel electrophoresis (Fig. 3). The pH 4.7-insoluble protein (pattern 1) contained considerable protein that did not move (glutenins) and only traces of the gliadins. In general, as pH increased, glutenins decreased and the gliadins increased. The pH 6.1-soluble protein (pattern 6) contained little or no glutenin and a high concentration of gliadins.

The decrease in glutenins and increase in gliadins as pH increased were accom-

TABLE I. PROTEIN CONTENT AND PROTEIN YIELD OF C.I. 12995 AND COMANCHE PROTEIN FRACTIONS OBTAINED BY PRECIPITATING, AT VARIOUS pH LEVELS, THE GLUTEN PROTEINS THAT HAD BEEN SOLUBILIZED IN 0.005N LACTIC ACID, TOGETHER WITH BAKING DATA OF RECONSTITUTED FLOURS THAT CONTAINED THOSE FRACTIONS

Flour and Gluten Fractions	Protein %	Part of Total Flour Protein %	Baking Absorption %	Mixing Time *min.*	Potassium Bromate Requirement *p.p.m.*	Loaf volume *cc.*
C.I. 12995						
Original flour			62.7	7	20	71
Crude gluten			63.8	6-1/4	20	74
One-step pptn.			....	....	....	....
All fractions			61.5	4-5/8	30	68
pH 4.7-insoluble	25.2	8.3	66.3	∞	20	22
pH 5.6-insoluble	76.5	20.2	64.8	6-1/2	20	67
pH 5.8-insoluble	78.1	22.4	62.8	5	30	75
pH 6.1-insoluble	79.2	23.9	61.8	3-1/8	40	81
pH 6.1-soluble	62.1	8.2	45.4	1/2	40	33
Starch + water-solubles	2.3	14.4	....	....	....	....
Total		94.4				
Comanche						
Original flour			62.0	4-1/2	25	78
Crude gluten			61.9	2-3/4	25	79
One-step pptn.			60.4	3-3/8	25	77
All fractions			62.4	2-3/4	25	72
pH 4.7-insoluble	16.3	4.8	69.1	∞	25	22
pH 5.6-insoluble	74.8	20.0	64.4	4-1/2	25	68
pH 5.8-insoluble	76.7	22.2	62.1	4-3/8	25	78
pH 6.1-insoluble	78.3	28.0	61.0	3-5/8	35	84
pH 6.1-soluble	61.7	11.1	47.2	1-1/8	40	39
Starch + water-solubles	2.3	14.2	....	....	....	....
Total		100.3				

panied by a consistently large decrease in mixing time and baking absorption, and, in general, an increase in oxidation requirement (Table I). Also, loaf volume increased with increasing pH. The loaf volume of the pH 6.1-insoluble fraction indicates that the ratio of gliadin to glutenin (pattern 5) was more nearly optimum than that in any of the other fractions or the crude gluten.

Water-Solubility of Flour Proteins

Because a considerable concentration of gliadins was found in the starch-gel electrophoretic pattern of the pH 6.1-soluble fraction, the water-solubility of flour proteins was studied by making consecutive water extractions of flour, when the initial ratio of flour to water was 1 to 4.8.

Yield and protein content of eight water-extractions of 100 g. RBS flour are given in Table II. Extracted protein increased from 6.5% for one extraction to 37.6% of total flour protein for eight consecutive extractions A to H.

Starch-gel electrophoretic patterns of A to D, the water-insoluble protein (WIP), and of starch (S) fractions are reproduced in Fig. 4. Patterns of E to H, WIP, and S fractions are reproduced in Fig. 5. All proteins in each of the fractions A to H migrated into the gel. The slowly moving bands of gliadins increased markedly with each consecutive extraction. The rapidly moving bands, on the other hand, decreased with each consecutive extraction. Water-insoluble protein and starch

TABLE II. YIELD AND PROTEIN CONTENT OF EIGHT WATER-EXTRACTIONS OF 100 g. RBS FLOUR

Original Flour or Fraction	Yield g.	Protein Content %	Part of Total Flour Protein Content %
RBS		12.8	
A	3.71	22.4	6.5
B	2.20	44.8	7.7
C	1.27	60.9	6.1
D	1.02	64.5	5.2
E	0.86	63.6	4.3
F	0.64	64.5	3.2
G	0.51	64.5	2.6
H	0.42	64.5	2.0
Total	10.63		37.6
WIP	10.28	59.4	47.7
S	80.04	2.5	15.6

fractions each contained the spectrum of proteins including glutenins, gliadins, and the rapidly moving bands. Of course, a relatively large quantity of the starch fraction was required to furnish the constant amount of protein (5 mg.) initially placed at the origin.

Water-soluble protein fractions (A to H), singly and in various combinations with the water-insoluble protein (WIP) and starch (S) fractions, were reconstituted into flours and baked into bread. Each reconstituted flour had a protein content equal to that of the original flour. Two or more WSP fractions were reconstituted according to their proportions in the original flour. Composition, loaf volume, baking absorption, and mixing time of the original and of each reconstituted flour are given in Table III. Relative size and crumb grain of the cut 10-g. loaves of bread are reproduced in Fig. 6.

Reconstituted flour (loaf 2) containing fraction A (rapidly moving proteins) gave a strikingly greater loaf volume (69 cc.) than that (51 cc.) of the reconstituted flour (loaf 7) containing fractions D to H (slower-moving, gliadin proteins).

TABLE III. LOAF VOLUMES, BAKING ABSORPTIONS, AND MIXING TIMES OF FLOURS RECONSTITUTED WITH THE STARCH RESIDUE AND WITH VARIOUS COMBINATIONS OF RBS WATER-SOLUBLE FRACTIONS AND THE WATER-INSOLUBLE PROTEIN FRACTION

Loaf No.	Composition of Reconstituted Flour	Loaf Volume cc.	Baking Absorption %	Mixing Time min.
1	Original flour	86	61.0	4
2	WIP + A	69	60.0	15-5/8
3	WIP + B	75	59.0	10-1/2
4	WIP + C	55	62.5	11
5	WIP + B,C	68	61.5	10-1/8
6	WIP + A,B,C	75	59.0	12-7/8
7	WIP + D,E,F,G,H	51	64.0	13-5/8
8	WIP + A,D,E,F,G,H	74	60.5	11-7/8
9	WIP + B,C,D,E,F,G,H	53	62.0	11-3/8
10	A,B,C,D,E,F,G,H	30	38.0	1-3/4
11	WIP	45	76.0	49
12	WIP + A,B,C,D,E,F,G,H	73	60.5	10-5/8

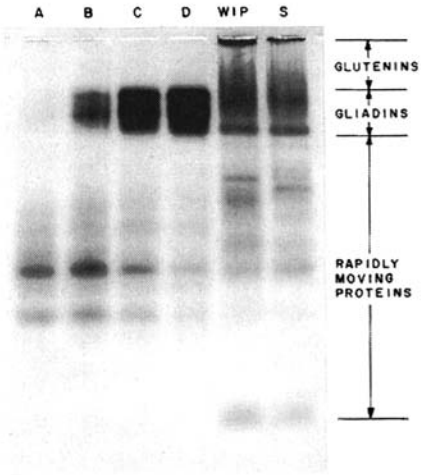


Fig. 4 (left). Starch-gel electrophoretic patterns of the A, B, C, and D water-soluble protein, the water-insoluble protein (WIP), and starch (S) fractions of RBS flour.

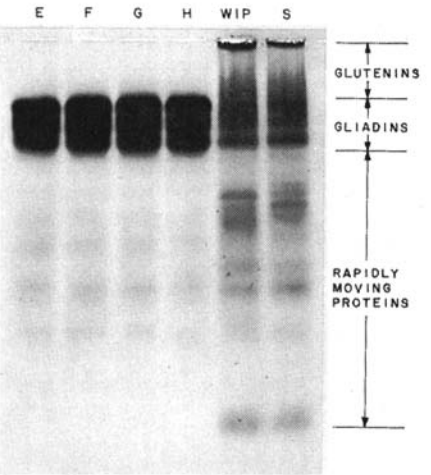


Fig. 5 (right). Starch-gel electrophoretic patterns of the E, F, G, and H water-soluble protein, the water-insoluble protein (WIP), and starch (S) fractions of RBS flour.

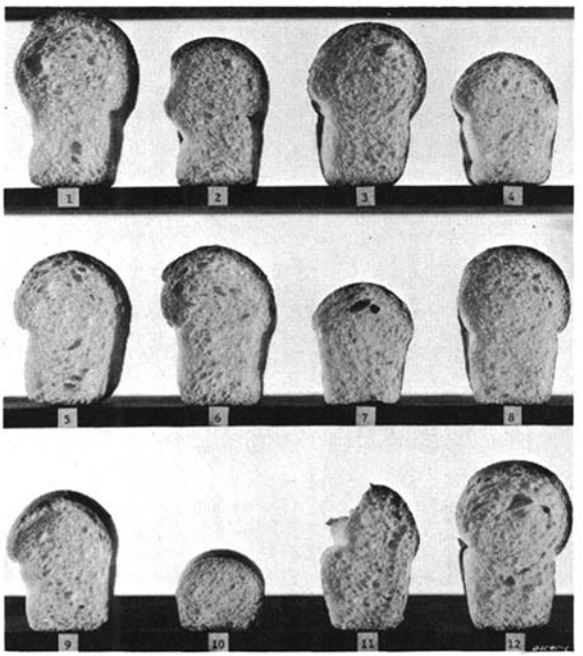


Fig. 6. Cut loaves of bread baked from flours that were reconstituted with the starch residue and various combinations of RBS water-soluble fractions and the water-insoluble protein fraction. Loaf numbers are identified in Table III.

Reconstituted flour containing all the water-soluble fractions, but without the water-insoluble fraction (Table III), had baking absorption, mixing time, loaf volume, and crumb grain similar to those of the pH 6.1-soluble fraction (Table I). Starch-gel electrophoretic patterns for the WSP fraction (composite of A to H, Figs. 4 and 5) and pH 6.1-soluble fraction (Fig. 3, pattern 6) were similar in protein composition.

The WIP fraction that remained after water-solubilization (eight extractions) had a mixing time of 49 min. Fractions A to H (loaf 10, Table III) were composed largely of gliadins, and had a short mixing time, only 1¾ min. Additional gliadins (not in table) were removed from the WIP fraction by 16 instead of eight water-extractions. Mixing time of the WIP fraction remaining after 16 water-extractions was 169 min., a value that, for all practical purposes, was directed toward infinity. Thus, as the gliadins were removed and the glutenins were concentrated, mixing time of the WIP fraction greatly increased. That evidence alone is not sufficient to conclude that either the gliadins or glutenins determine mixing time, because it is likely that other factors, including the gliadin-glutenin as well as lipid-protein interactions, are involved. Mattern and Sandstedt (5) stated that "the principal factor responsible for determining the mixing requirement of wheat flour is water-soluble." Smith and Mullen (6) showed that both the water-soluble and residue proteins were involved.

Loaves 10, 11, and 12 illustrate the protein interaction between the WSP and WIP fractions. Either one is ineffective without the other. In the absence of protein interaction, a loaf volume intermediate between 30 and 45 cc. would have been obtained instead of 73 cc. An example of protein interaction within the soluble-protein fractions includes A (69 cc.) vs. D, E, F, G, H (51 cc.) vs. A, D, E, F, G, H (74 cc.).

Mixing times of flours reconstituted from water-extracted fractions were generally longer than those of flours reconstituted from lactic acid-extracted fractions. Longer mixing times can be attributed to fractionating techniques and separation into protein systems that required long mixing to re-establish the protein association and interaction that existed after the original flour was wetted and mixed. Data in the table below demonstrate, to some extent, that virgin protein association and interaction were re-established more rapidly by bringing the WIP

Effect of Treatment (see text) on Mixing Time and Loaf Volume

Sample and Reconstituting Technique	Mixing Time *min.*	Loaf Volume *cc.*
WIP-S + WSP	9 1/4	77
Wet WIP-S + WSP	9 1/8	76
[Wet WIP + WSP] LA + S	6 5/8	76

and WSP fractions together in 0.005N lactic acid (LA), followed by stirring, adjusting to pH 6.1, and lyophilizing prior to baking. Mixing time was shortened from 9¼ to 6-5/8 min. by acid treatment.

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